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PAPER CHROMATOGRAPHY OF PLANT PROTEINS¹

APOLINARIO R. CARANGAL, JR., ADELINA M. CATAMBAY,
AND ELVIRA B. MANZANILLA

WITH ELEVEN TEXT FIGURES

Protein solutions of egg albumen and coconut meat, mungo beans, peanut, and crude latices of *Carica papaya* Linn. and *Ficus sp.* were analyzed by paper partition chromatography. The chromatographic method may be of value in the diagnosis of plant diseases affecting protein metabolism, in the isolation of enzymes, and in peptization studies of plant proteins. Detergents were found to be effective in preventing the movement of certain proteins.

Proteins are purified and separated from one another by techniques which are quite different from those used with smaller organic molecules. The usual methods of protein separation are by heat denaturation, by changing the ionic strength and dielectric constant, and by using protein precipitants such as salts of heavy metals, organic acids, colloidal acids, and bases. These protein precipitants are extensively used in clinical laboratories. In recent years electrophoresis has proved to be effective for separating protein mixtures and particularly for studying their composition and testing for the homogeneity of protein systems. However, this method requires elaborate and expensive apparatus.

Partition chromatography of amino acids, the building units of proteins, has been widely developed. To some extent peptides have also been separated by paper chromatography by Knight (1951), Heyns and Walker (1952), Boulanger, Biserte, and Courtot (1952), and other workers. Franklin and Quastel (1949) initiated the separation of some crystalline protein preparations and enzymes by paper chromatography. Since then Franklin, Quastel, and Van Straten (1951), Zimmerman and Khudas (1953), Berlingozzi, Rapi, and Dettoni (1953) have applied similar methods to the separation of serum proteins. Franklin and his associates (1951) have stressed its application to clinical research for diagnostic test.

In this study some natural protein systems from plants were subjected to paper partition chromatography. Mungo beans and peanuts were selected because of their relatively high protein content, crude latices of *Carica papaya* and *Ficus sp.* because of their enzyme systems, and coconut because of the need for further studies on the cadang-cadang disease. The results of various works on developing solvents and the use of detergents and protein-staining reagent were applied in the chromatographic method.

¹ Central Experiment Station Contribution No. 1822. Prepared in the Department of Agricultural Chemistry.

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EXPERIMENTAL PROCEDURE

Protein solutions. Separated egg albumen and freshly gathered latices from the fruits of *Carica papaya* and *Ficus sp.* were diluted with water to facilitate spotting on filter paper. A mature coconut was grated and the cream was expressed by hand. The proteins of defatted peanuts and mungo beans were extracted with 0.4 M NaCl after the peanuts and mungo beans had been ground in the Wiley mill. The salt extracts were analyzed. Undoubtedly the proteins of the coconut, peanuts, and mungo beans were not completely extracted. Mixtures of coconut and egg albumen and mungo and egg albumen were prepared.

Paper chromatography. The protein solutions were subjected to two-dimensional chromatography by the capillary ascent on 14 centimeters square of Schleicher & Schull No. 604D filter paper. Aqueous solutions (0.1 M) of sucrose (first solvent) and sodium potassium tartrate were used as solvents. The chromatograph was run in glass aquariums at 25 to 30°C. Local earthenware may also be used.

The samples were spotted repeatedly on one corner of the filter paper with micropipettes. The amount placed on the paper was measured qualitatively with the protein-staining reagent. The spots were less than 1 centimeter in diameter. After drying in air, cylinders were formed and developed with the solvent in Petri dishes. The chromatograms were dried in air after the solvent had risen to about ten centimeters from the point of application. The development with the second solvent was done in the same manner and the chromatograms were also dried in air. The first solvent traveled uniformly, but the second solvent was quite irregular.

The proteins were visualized by staining with brom phenol blue reagent (Geschwind and Choh, 1952). This reagent consists of 0.1 per cent brom phenol in 95 per cent ethyl alcohol previously saturated with HgCl_2 . The dried chromatograms were dipped in the reagent or brushed lightly with the reagent. After about five minutes the excess reagent was washed away thoroughly with running water and the chromatograms were dried between filter or toilet paper at room temperature. The removal of the excess reagent left the protein-containing areas a green-blue color. The protein-staining reagent of Tauber and Petit (1952) containing eosin and methyl orange may also be used to some advantage if ultra-violet light (Mineralight) is available. The chromatograms that were obtained of the different proteins are shown in figures 1 to 6.

DISCUSSION OF RESULTS

Chromatographic method. The analysis took place three to four hours after the application of the protein on the filter paper. Difficulties were encountered in the flow of the second solvent. The solvent front was irregular unlike that of the first solvent of organic solvents. This non-uniform flow of solvents was perhaps due to the



Fig. 1.—Two-dimensional chromatogram of mungo bean protein.

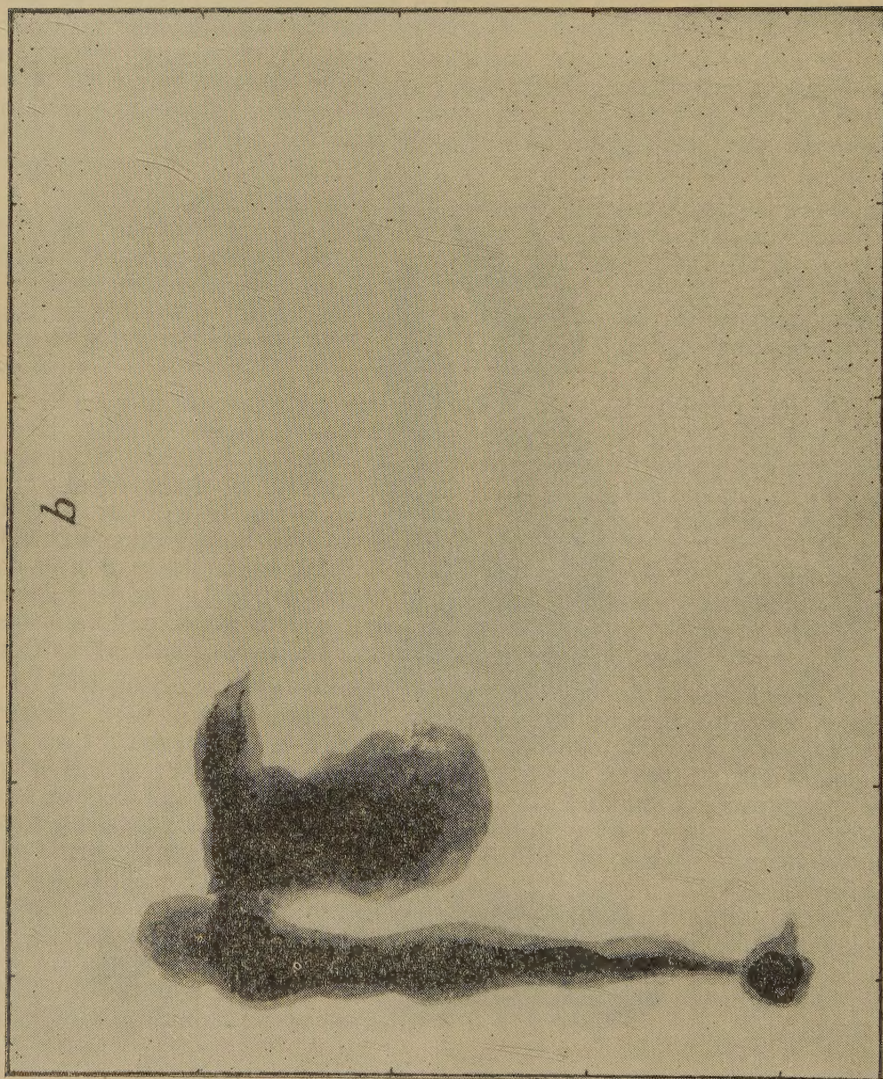


Fig. 2.—Two-dimensional chromatogram of peanut protein.

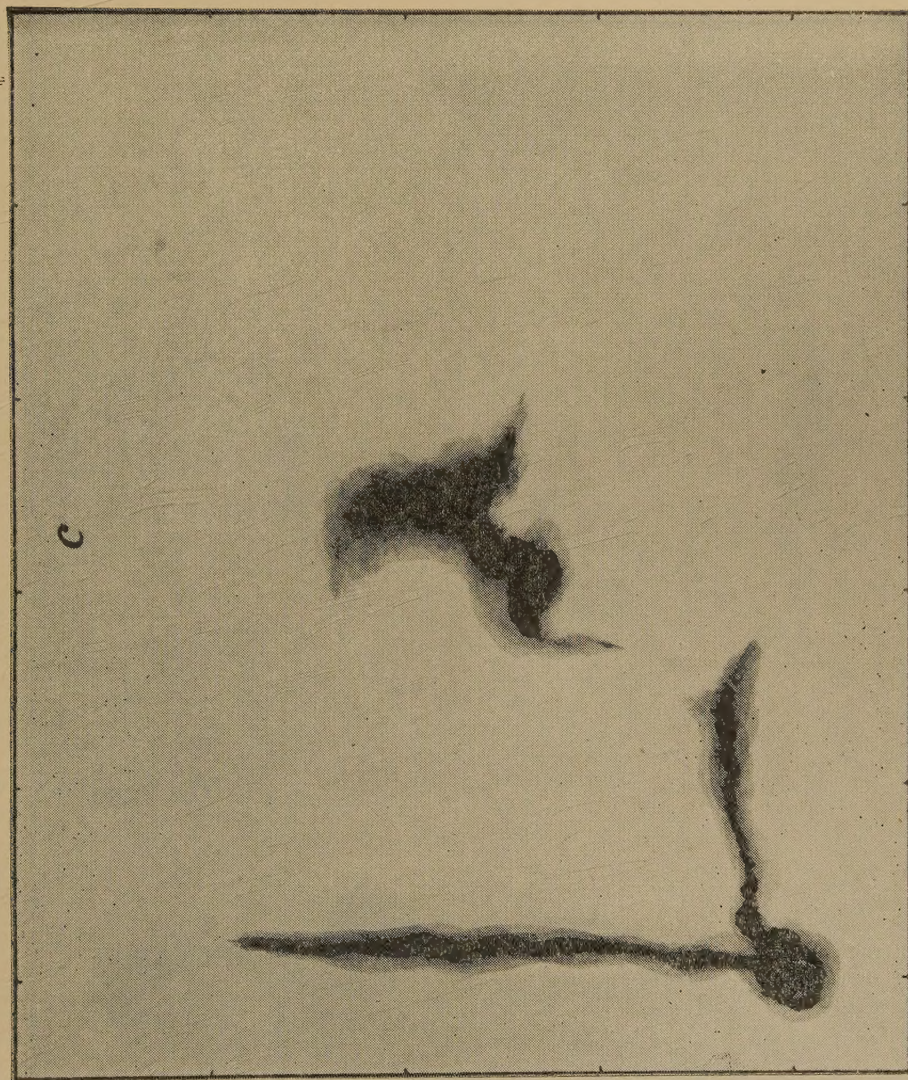


Fig. 3.—Two-dimensional chromatogram of coconut protein.

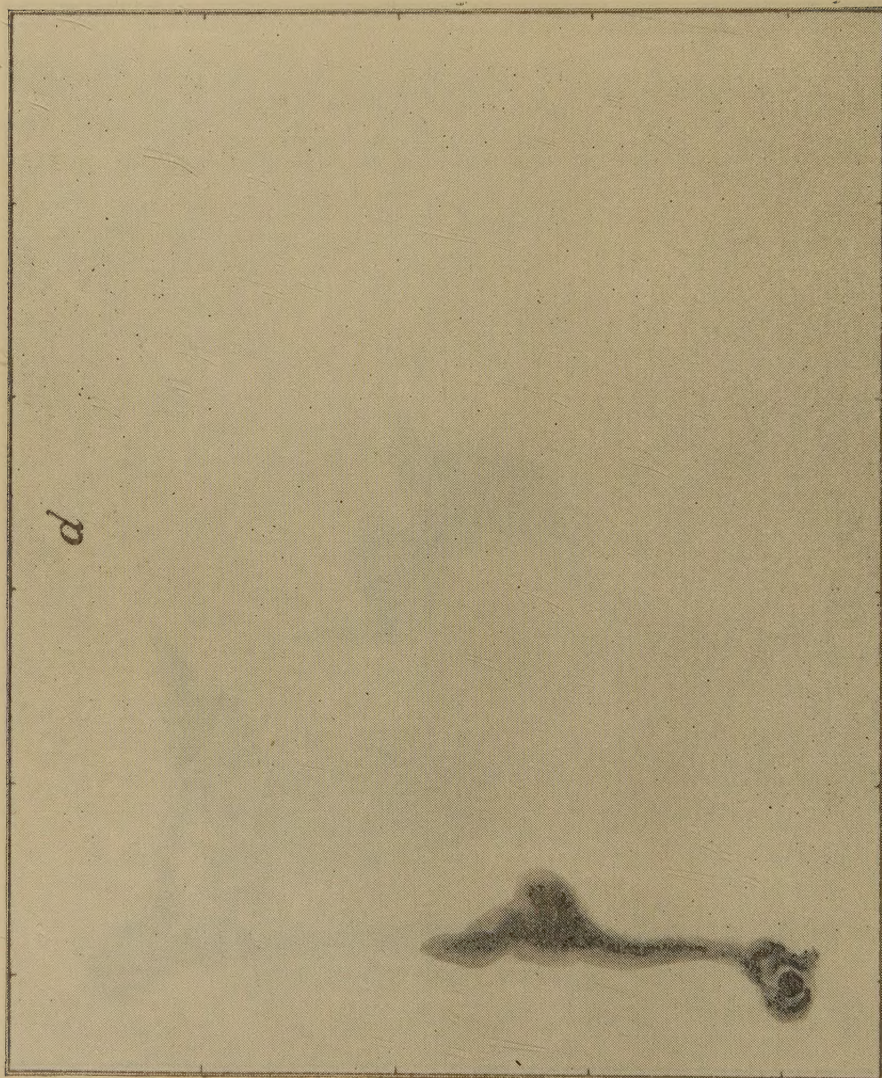


Fig. 4.—Two-dimensional chromatogram of egg albumen.

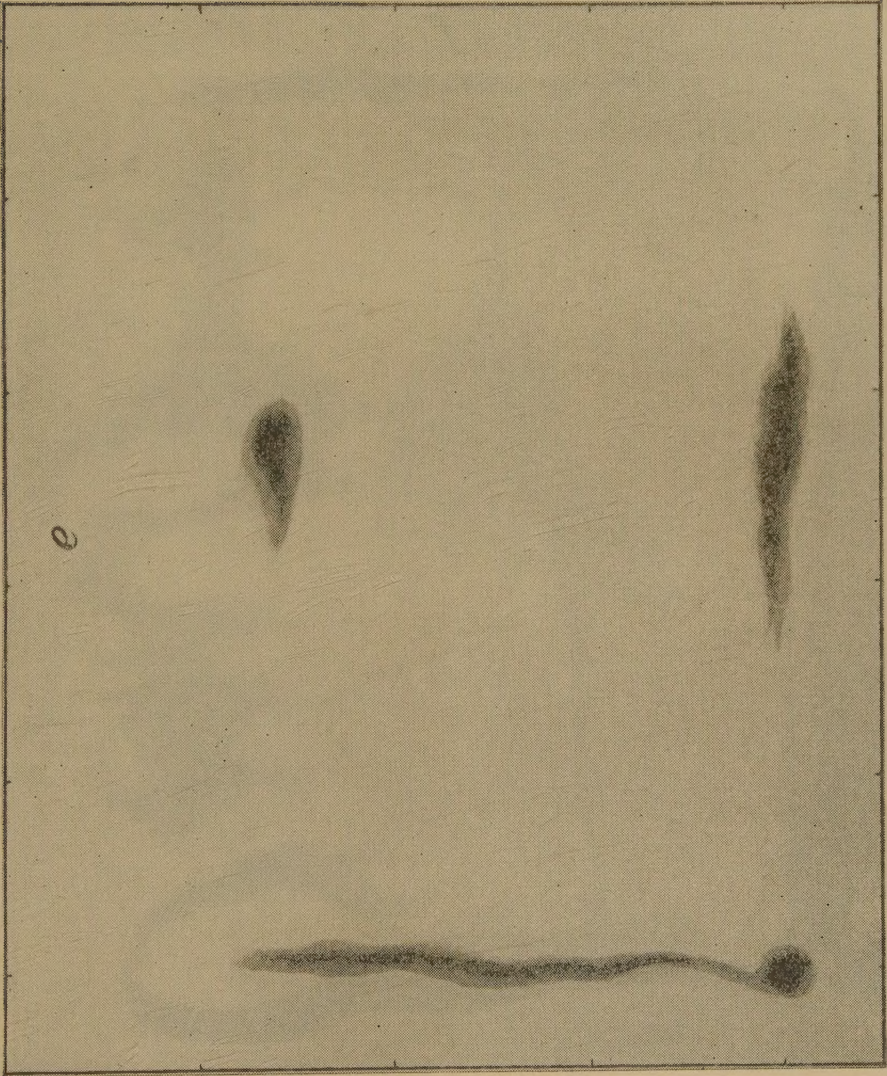


Fig. 5.—Two-dimensional chromatogram of latex of *Ficus* sp.

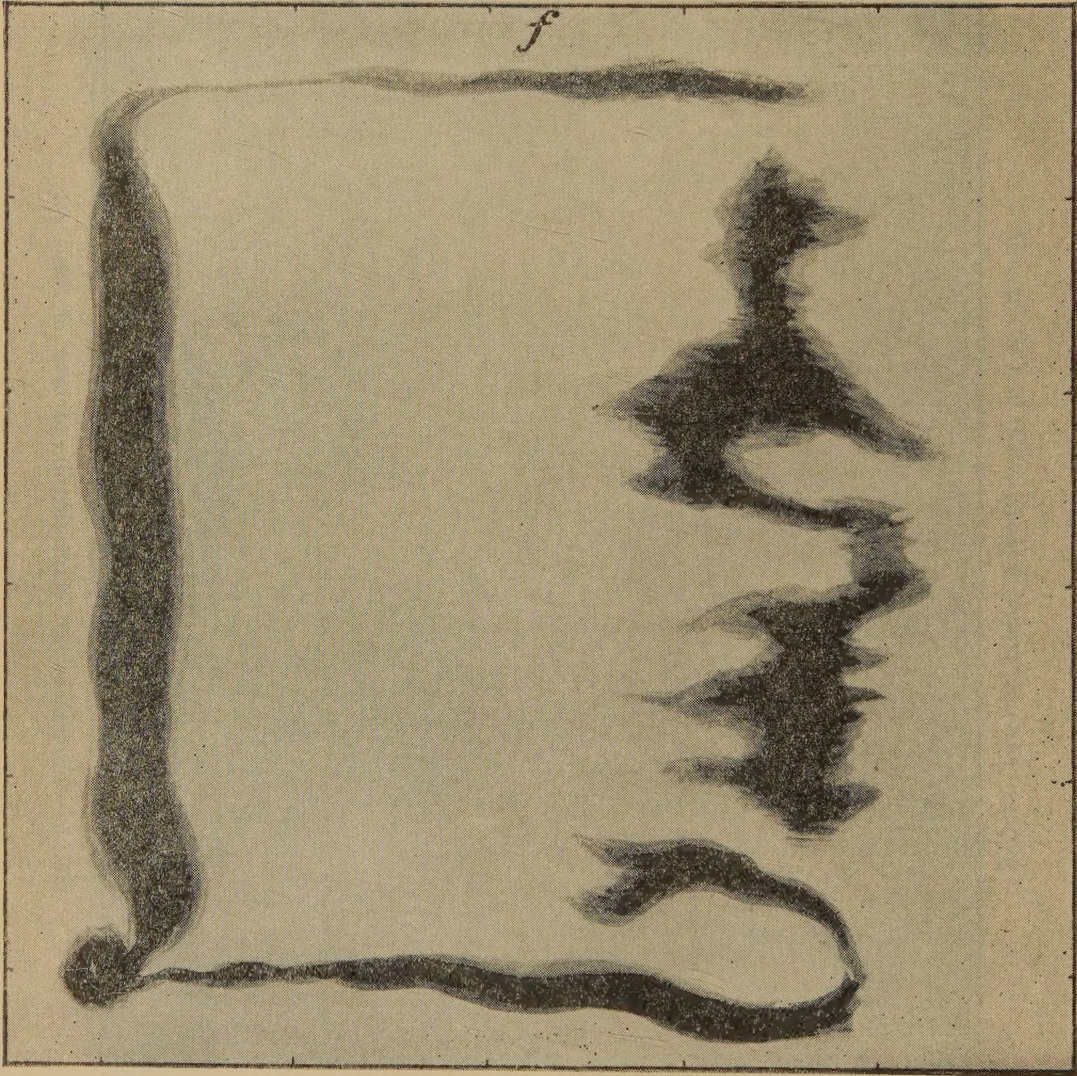


Fig. 6.—Two-dimensional chromatogram of latex of papaya.



Fig. 7.—Two-dimensional chromatogram of coconut-egg albumen protein system.



Fig. 8.—Two-dimensional chromatogram of mungo-egg albumen protein system.

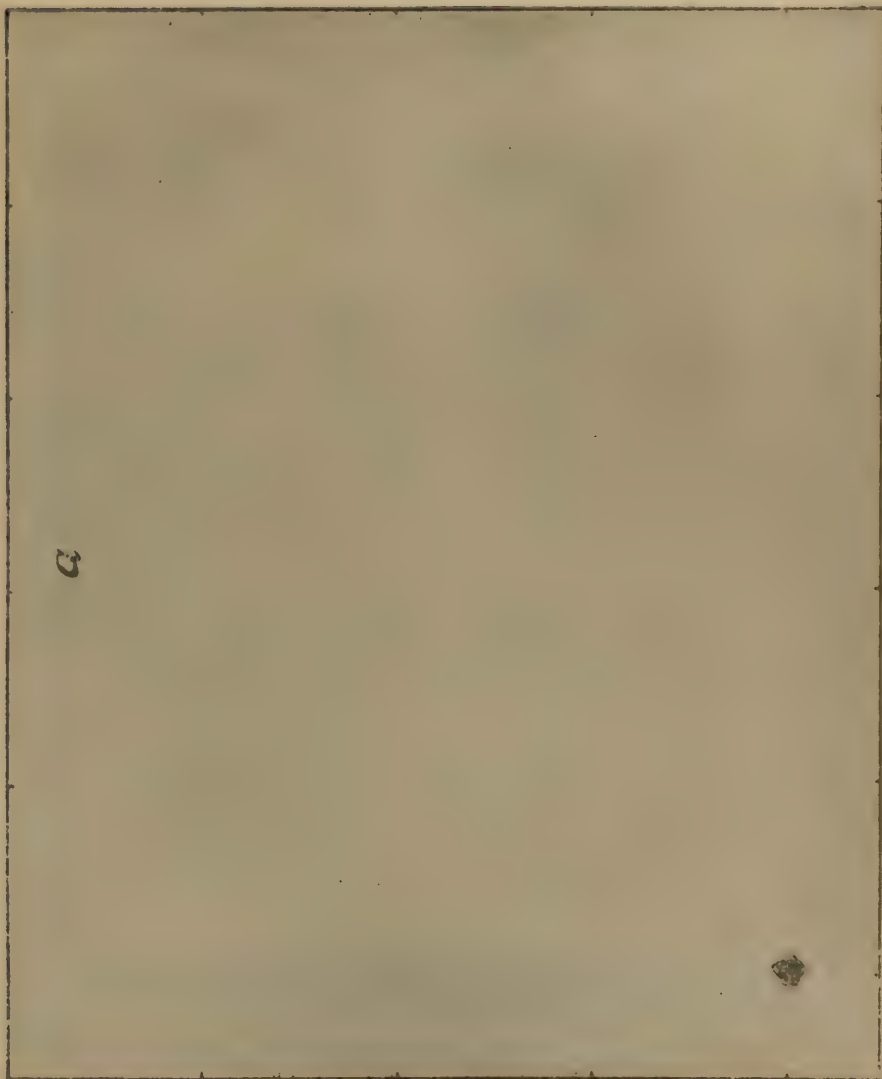


Fig. 9.—Effect of the addition of detergent ("Dreft") on the chromatographic pattern of egg albumen.

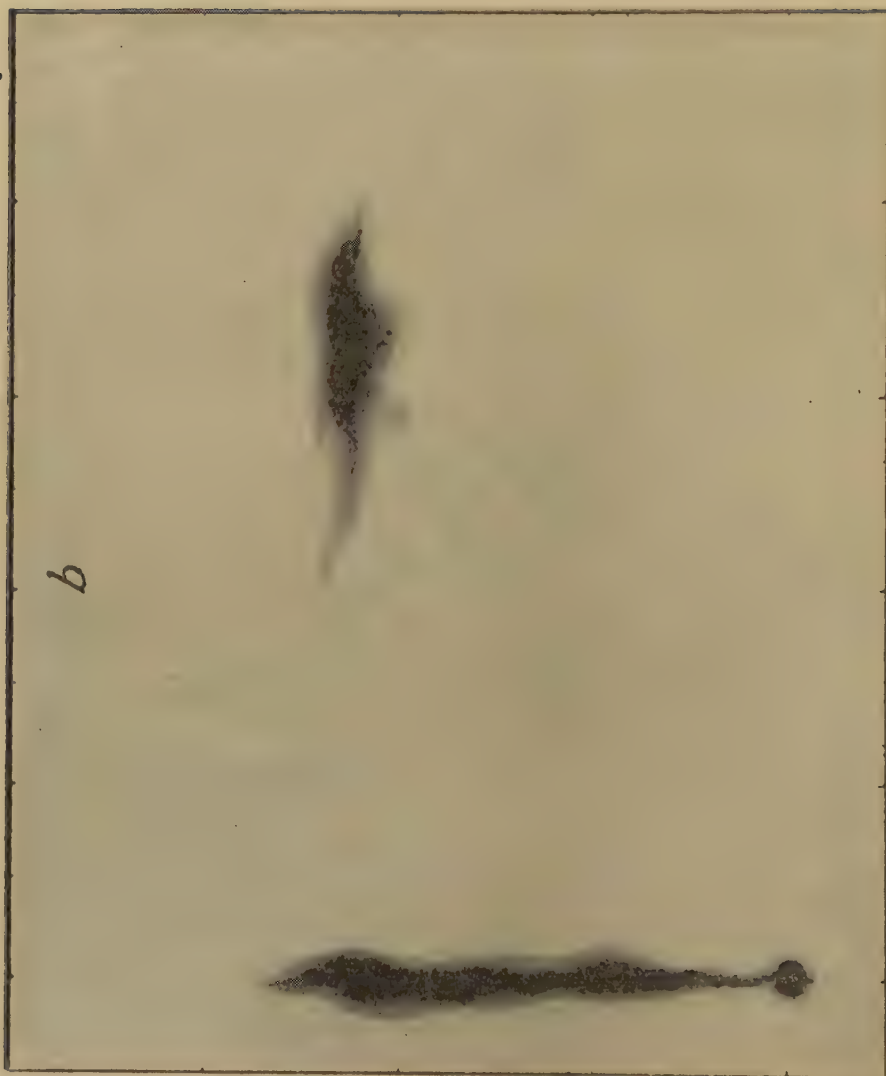


Fig. 10.—Effect of the addition of detergent on the chromatographic pattern of mungo beans.



Fig. 11.—Effect of the addition of detergent on the chromatographic pattern of coconut.

change in the nature of the paper as a result of the absorption of some amounts of sucrose. Undoubtedly the movement of the moving protein fraction was affected in the second solvent. However, reproducible chromatographic patterns were obtained with the protein solution studied.

The brom phenol blue reagent used by Geschwind and Choh (1952) for staining peptides on paper was found advantageous. Other dyes are used for staining proteins. Tauber and Petit (1952) have developed a reagent containing eosin and methyl orange. The outlines of the pattern are checked with the aid of ultraviolet light. Amido black has also been used by Berlingozzi, Rapi, and Dettoni (1954), and solvay purple by Jones and Michael (1950). Franklin and his associates (1951) first converted their protein to protein-hemin complexes and identified their position by streaking the paper with benzidine and hydrogen peroxide. Thus, the protein system was not actually being separated. Its use was limited to those proteins that would combine with hemin.

Chromatographic patterns of the protein systems. With low molecular weight compounds such as sugars, amino acids, and simple peptides, multiplicity of spots shows a multiplicity of molecular species. Franklin and his associates (1951) refer to "pattern" for complex mixtures like serum and snake venom and to "separation" when much less complex protein mixtures are considered. Hall and Wewalka (1951) criticized the procedure of Franklin and his co-workers but concluded that the method was perhaps practicable with proteins of quite dissimilar nature although not with complex mixtures. Using hemin as marker of the protein systems being studied makes the mixture more complex. Hemin was not used in this study, but the protein solutions were chromatographed as prepared. It is much safer to assume chromatographic patterns rather than separation at this stage of the work because of lack of evidence.

The chromatographic patterns of the protein systems revealed separated fractions which are reproducible (figs. 1-6). There was no complete separation, however. With mungo proteins which revealed two major fractions, Djang, Ball, and Lillevick (1953) found four fractions of different electrophoretic behaviors in the salt (0.4 M NaCl) extract of mungo beans. Preliminary experiments with proteins of mungo extracted with water, NaCl, and NaOH have shown chromatograms of different patterns.

The latices of papaya and Ficus also revealed some separated fractions which could be tested for their enzymatic activities as was done by Franklin, Quastel, and Van Straten (1951) with urease and by Giri and Prasad (1951) with amylase and trypsin. Thus, papain and ficin may possibly be identified in those separated fractions.

Mixtures of proteins (coconut-egg albumen and mungo-egg albumen) were also tried (figs. 7-8). Interactions between these protein systems may occur and thus can be revealed by paper chromatogra-

phy. Their chromatograms showed no differences in patterns when the proteins were chromatographed separately.

Unlike amino acids which are easily identified on the chromatograms by their R_f values and characteristic color reactions, proteins and enzymes of complex protein systems separated by this method will have to be analyzed further. Amino acid analysis, electrophoresis, enzymatic activity, and the use of proper solvents at different pH may be used in conjunction with paper chromatography to furnish proofs of differences in molecular species and homogeneity of the fractions separated.

Effect of the detergent. Franklin and Quastel (1950) showed that certain polyhydroxy emulsifying agents increased the number of fractions into which serum could be divided. The detergent "Dreft" (sodium lauryl sulfate) was used in this work. The effect of this detergent seems to depend upon the type of protein system. Thus, the chromatographic pattern of the mungo proteins did not differ when the detergent was added, but that of egg albumen and detergent did not move at all (figs. 9-11). On the other hand the chromatogram of coconut with detergent was different from the chromatogram of coconut alone, showing that detergents may be used to some advantage to prevent the movement of certain proteins.

APPLICATIONS

Although no actual proof has been presented to show that separation may be achieved, analysis by paper chromatography may be of value in studies of plant diseases affecting protein metabolism. Its use as a diagnostic test for abacá diseases and the "makapuno" character and cadang-cadang disease of coconut will be investigated. Franklin and his associates (1951) have applied this method to normal and pathological plasma.

The nature of the patterns obtained with the crude latices of papaya and Ficus suggests the use of this method in the isolation of enzymes. Peptization studies of plant proteins may also be followed by chromatographic analysis of the solubilized proteins.

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BIOCHEMICAL STUDIES ON ABACÁ MOSAIC VIRUS AND BUNCHY-TOP OF ABACÁ VIRUS: II. THE OR- GANIC ACIDS OF ABACÁ ¹

APOLINARIO R. CARANGAL, JR., ADELINA M. CATAMBAY,
AND ELVIRA B. MANZANILLA ²

Oxalic, acetic, and lactic or succinic acids were found by chromatographic method in the roots, stems, and leaves of normal and diseased abacá plants. In all parts of the plant, oxalic acid was the predominant acid, followed by acetic acid. Abacá mosaic virus infection does not seem to cause abnormal metabolism of the organic acids through the formation of acids other than those found in healthy plants.

Organic acids have already been recognized as substances of primary importance in carbohydrate, protein, and fat metabolism as well as in photosynthesis. Studies on organic-acid metabolism have been handicapped by the lack of analytical procedures. Macro-quantitative analyses, such as fractional precipitation, have been superseded by the much superior chromatographic method for the separation and tentative identification of the commonly occurring acids.

No analysis has ever been made of the organic acids of abacá as far as the writers know. Preliminary work has shown that the pH of the aqueous extract of the leaves of healthy seedlings range from 6.4 to 6.6, and that of mosaic-infected plants, from 5.6 to 6.3. There are reports that virus infection tends to lower the pH of the plant. Bolas and Bewley ³ found that the acidity of aqueous extracts of infected leaves is greater than that of healthy ones. The sap of spiked sandal leaves seems to have a lower pH than normal leaves.⁴ According to Rouzinoff ⁵ pH values of the juices inside the stems and tubers indicate that the juices are more acidic in plants with leaf roll than in normal plants.

The acid content of abacá was studied by the writers to obtain additional information regarding the metabolism of the plant and perhaps to correlate metabolism with virus diseases like mosaic and bunchy-top. Knowledge of the acid content of abacá may also serve as a guide in fertilization work since the acids partake in the anion-cation equilibrium of the plant sap.

In this study the organic-acid content of the roots, stems, and leaves of normal and mosaic-infected abacá seedlings were analyzed by chromatographic method in silica gel column.

¹ Central Experiment Station Contribution No. 1823. Prepared in the Department of Agricultural Chemistry.

² The writers thank Dr. G. O. Ocfemia, Head of the Department of Plant Pathology, and Dr. D. L. Umali of the Department of Agronomy for the abacá plants used in this study.

³ B. D. Bolas and W. F. Bewley, *Nature*, **126**, 471 (1930).

⁴ A. V. V. Iyengar, *Jour. Indian Inst. Sci.*, **11A** Pt. 9, 97 (1928).

⁵ P. G. Rouzinoff, *Morbi Plantarum, Leningrad*, **19**, 148 (1930).

EXPERIMENTAL PROCEDURE

The stems, roots, and leaves of one- to two-year-old abacá seedlings were cut into small pieces and dried in the oven at 80°C. for 24 hours. The dried samples were then ground and stored in the dessicator.

The organic-acid fractions for chromatographic analysis were prepared by extraction with ether and dried as sodium salts. The ground dried samples were triturated with 1 to 2 ml. of 4N concentrated sulfuric acid, and the mixture was taken up with silica gel. The samples were extracted with ether (previously washed with 0.1N KMnO_4) for 12 to 24 hours. The acids were recovered from the ether extract as sodium salt by titration of the extract with 1.0 N NaOH until alkaline to phenol red. The alkaline extract was separated and dried on the hot plate.

The silica gel column, 14 mm. inside diameter $\times$ 11 cm., was calibrated by using acetic, formic, lactic, oxalic, and citric acids to determine its displacement volume with known volumes of increasing concentrations of n-butanol in chloroform according to the method of Bulen, Varner, and Burrell.⁶ Previous works have shown quantitative recoveries of 85 to 98 per cent of these acids in concentrations of 1 to 5 mg. Based on these calibration experiments, the organic-acid fractions were separated on the column by using butanol-chloroform as solvent, and the separated fractions were titrated with 0.01N NaOH. The titration values were plotted against the fraction number and the concentration of the acids was expressed in milliequivalents after correction for the blank. The separated acids were identified by their position in the chromatogram previously prepared in the calibration experiments.

DISCUSSION OF RESULTS

The organic acids which were found present in the roots, stem, and leaves of healthy abacá were acetic, oxalic, and an unidentified acid belonging to the lactic group according to the chromatogram of Bulen, Varner, and Burrell. This acid may be lactic or succinic acid. It could not be identified by the formation of its derivatives because of the small amount that was recovered, as shown below:

PLANT SAMPLE	PART OF THE PLANT	TRIAL	ORGANIC ACIDS Acetic	(milliequivalents Lactic or Succinic	$\times 10^2$) Oxalic
Healthy	Roots	I	19.2	1.7	95.4
		II	34.6	2.1	144.2
	Stems	I	31.3	4.7	59.7
		II	23.4	2.4	63.4
	Leaves	I	48.3	1.2	121.5
		II	21.8	2.7	101.0
Mosaicked	Roots	I	20.2	1.9	43.8
		II	26.4	1.5	58.4
	Stems	I	13.9	1.0	50.3
		II	17.4	1.4	47.3
	Leaves	I	9.9	0.6	11.8
		II	18.2	2.4	42.5

⁶ W. A. Bulen, J. E. Varner, and R. C. Burrell, *Analyt. Chem.* **24**, 187 (1952).

The acids in the different parts of the plants did not differ in nature. Of these acids, oxalic acid was the most predominant, followed by acetic acid, in all the parts of the plant analyzed. Oxalic acid accounted for as high as 82 per cent, and acetic acid 32 per cent, of the total acids based on the number of milliequivalents.

Although oxalic acid is not involved in the Krebs cycle, together with acetic acid it plays an important role in the maintenance of cation-anion equilibrium of the plant sap. Furthermore, the presence of oxalic acid is very much related to calcium fertilization. The formation of calcium oxalate ties down the calcium ion. These facts should be considered in connection with the calcium fertilization of abacá, and the effect of different levels of calcium on abacá with respect to oxalic acid should be further studied. Iljin⁷ has made extensive analyses of 10 plant species and shown that the total acids, malic and citric acids, are correlated with the calcium content, especially in plants growing on chalky soil.

The presence of certain organic acids and respiratory enzymes has been taken as evidence that plant and animal respirations follow the same path. As far as is known, not all the acids of Krebs's cycle occur in a plant. Of the acids found in abacá, succinic or lactic and acetic acids are involved in the Krebs cycle. Acids like ketoglutaric, oxaloacetic, and pyruvic acids, which are related to nitrogen metabolism by transamination reactions, were not found, probably because they were too small in amount to be detected by the analytical procedure used or because they were readily converted to other intermediates of the Krebs cycle or to amino acids by transamination. Thus, organic-acid metabolism may be indirectly related to virus formation if the presence of transaminases in abacá can be proved.

In preliminary experiments to compare the organic-acid contents of virus-infected and healthy plants, no abnormal organic-acid metabolism was found in terms of other acids being formed as a result of mosaic infection. The same acids were found in healthy and diseased plants. Perhaps virus infection may affect organic-acid metabolism by changing the level of the acids, as shown by previous findings that virus-infected plants are more acidic than normal ones. To prove that virus infection does affect the level of the acids, plants grown under similar conditions of fertilization will have to be analyzed. Calcium, potassium, and nitrate nitrogen are reported to favor the accumulation of acids, and phosphorus and ammonium nitrogen to repress organic-acid formation.

⁷ V. S. Iljin, *Flora*, 37, 265 (1944).

A COMPARISON OF THE RAPID MICROCHEMICAL SOIL TESTS AND SEEDLING TEST IN DETERMINING THE AVAILABLE PHOSPHORIC ACID AND POTASH CONTENTS OF LIPA CLAY ¹

CONCEPCION L. VALERA

WITH THREE TEXT FIGURES

The Neubauer test using rice is of no value for measuring the root soluble phosphorus in Lipa clay loam, there being no marked difference between the phosphorus removed from the soil and that contained in the check seedlings grown in quartz sand. Conducted under uncontrolled temperature, it satisfactorily extracted more available potassium than the rapid microchemical soil test of Wolf and Ichisaka. All the paddies sampled contained the same quantities of phosphoric acid and potash. In the determination of potassium, results from the perchloric acid method of Reiser were found to be in close agreement with those from the rapid chemical plant test of Wolf and Ichisaka. The results of the perchloric acid method were more uniform, however.

Many methods have been developed and used to measure the available phosphoric acid and potash in soils. The seedling test of Neubauer has received much investigational work in foreign countries, especially in Germany, and has been found to be an accurate and reliable method if carefully followed. The microchemical soil tests, which are simpler and take less time to perform, are however rapidly replacing the classical and more conventional methods. This study, conducted in the Soils Department Laboratory from April, 1953, to February, 1954, compares the reliability of the microchemical soil tests and the seedling test as indicators of fertilizer needs under Philippine conditions.

MATERIALS AND METHODS

Soil. Lipa sandy clay loam collected from a lowland rice field in Bucal, Calamba, Laguna, was used in this experiment. The land has been grown to barit (*Leersia hexandra* Sw.) for many years. It is cropped twice a year. Since 1951, it has received ammonium phosphate (16-20-0) at the rate of 100 kilograms to a hectare every planting season.

The surface soil is brown and about twenty-one centimeters deep. It is friable and pulverizes easily when dry and is plastic and slightly sticky when wet. Physical and chemical analyses show it to have the following properties: sand, 46.31 per cent; silt, 24.36 per cent; clay, 29.33 per cent; moisture content, 10.31 per cent;

¹ Central Experiment Station Contribution No. 1824. Thesis presented for graduation, April, 1954, with the degree of Bachelor of Science in Agriculture from the College of Agriculture. Prepared in the Department of Soils under the direction of Dr. Nicolas L. Galvez.

water-holding capacity, 46.31 per cent; pH, 6.14; organic matter, 3.89 per cent; nitrogen, 0.11 per cent; nutrients held in reserve, K_2O , 1.23 per cent; P_2O_5 , 0.66 per cent; Na_2O , 0.58 per cent; CaO , 0.99 per cent; and sesquioxides, 15.15 per cent. The subsoil is black when moist and shows the following textural grade: sand, 66.21 per cent; silt, 19.03 per cent; and clay, 14.76 per cent.

The Neubauer seedling test. The nonbearded Thailand variety of rice, 98 per cent viable, was used as indicator. It is a standard lowland variety which matures in 119 days. The seeds were soaked in water to show up the unfilled ones, and the healthy grains were carefully selected and weighed. The average weight of 100 grains was 2.4964 grams. The seeds were not given any treatment.

Fifteen paddies were sampled by compositing 25 randomized borings from each paddy. The soil was air-dried, passed through a two-millimeter sieve, and thoroughly mixed. Each soil composite was replicated four times. A mixture of 100 grams of air-dried soil and 50 grams of nutrient-free quartz sand² was placed in each glass dish which had a diameter of about 11 centimeters and a height of 7 centimeters. The surface of the medium was leveled and moistened evenly with 20 ml. of rain water. Another layer of 150 grams of quartz sand was added, leveled, and moistened with 40 ml. of rain water.

With forceps, 100 of the selected rice seeds were planted equidistantly in each dish on June 26, 1953. A glass tube 1 centimeter in diameter and 7 centimeters in height was inserted upright in the center. This facilitated watering and aeration during the period of growth. The seeds were covered with 100 grams of quartz sand which was then uniformly moistened on the surface with 20 ml. of rain water. The dishes were weighed and properly marked. Four blanks were prepared using quartz sand alone for the culture medium. The original weights of the dishes were maintained daily by the addition of rain water. The cultures were covered with thick cardboard until the seedlings were tall enough to touch the cover.

After 20 days of growth, the seedlings were harvested and the roots washed free of soil with running water. A .5-millimeter sieve was used to catch all the fine roots, ungerminated seeds, and loose seed coats. The whole seedlings were dried, dry-ashed, and extracted with 10 per cent HCl. The amount of potash was determined in 50-ml. aliquot by the perchloric acid method of Reiser, and the phosphoric acid by the Wolf and Ichisaka³ rapid chemical tests.

The rapid microchemical soil tests. A 12.5-gram sample of field soil was placed in an Erlenmeyer flask. One-half teaspoonful of activated charcoal and 25 ml. Morgan's Universal extracting solution were added. The flask was shaken for 1 minute after which the contents were filtered.

² Imported from the Ottawa Silica Company, Ottawa, Illinois.

³ Benjamin Wolf and Vernon Ichisaka, *Soil Sci.*, 64, 227 (1947).

In an electrophotometer vial was placed 2 ml. of soil extract, diluted to 5 ml. with Morgan's Universal extracting solution. Then 3 ml. of formaldehyde, 0.25 ml. of .25 per cent gum arabic, and 2 ml. of potassium cobaltinitrite solution were added. The contents were mixed by rotating the tube and were allowed to stand for 5 minutes. With a pipette at a constant height above the vial, 10 ml. of isopropyl alcohol was directly added to the liquid. The vial was immediately stoppered and inverted three times. It was allowed to stand for 15 minutes before potassium readings were taken with the Klett-Summerson photo-electric colorimeter.

A 5-ml. portion of soil extract was diluted to 20 ml. with Morgan's Universal extracting solution. Then 4 ml. of ammonium molybdate was stirred in before 2 ml. of amino-naphthol-sulfonic acid was added. The contents were stirred and allowed to stand for 15 minutes before phosphorus readings were taken with the Klett-Summerson photo-electric colorimeter.

RESULTS AND DISCUSSION

Cultural observations. The seeds in the sand-soil medium germinated three to four days after planting; those planted in quartz sand alone germinated four to five days after. The cultures were exposed to sunlight every morning to prevent the rice seedlings from growing tall and pale with weak stems and undeveloped leaves. The seedlings grew fast during the first 10 days. They were all upright; leaves were green and developed.

The check seedlings were shorter and had narrower leaves than those grown in the sand-soil medium (figs. 1 to 3). On the eleventh day, browning of the tips of the leaves of the check seedlings was

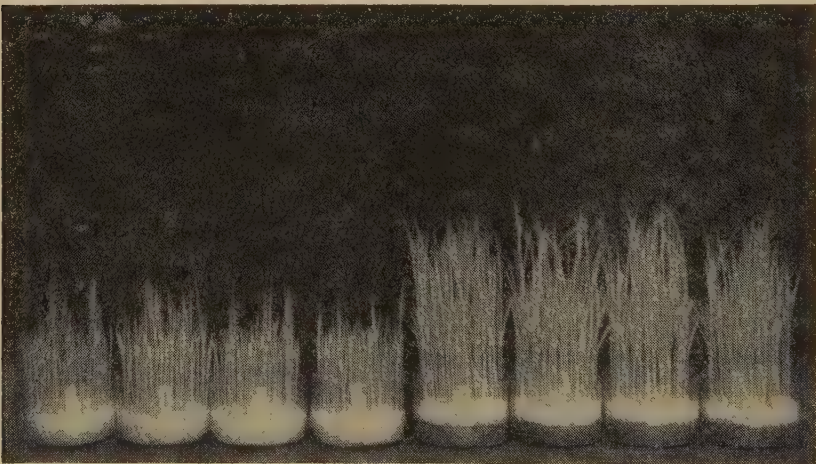


Fig. 1.—Ten-day-old rice seedlings showing check at left and soil test at right. (Photographed by the Photographic Division, College of Agriculture.)

observed. The seedlings became thin but continued to grow at a slow rate. On the fourteenth day, the same symptoms were observed on the seedlings grown in the sand-soil medium. These symptoms are indications of potash deficiency. Three days after, most of the leaves turned yellow and the outer ones started to dry up. Phosphorus deficiency symptoms were not observed on the check seedlings because of the lack of potassium.

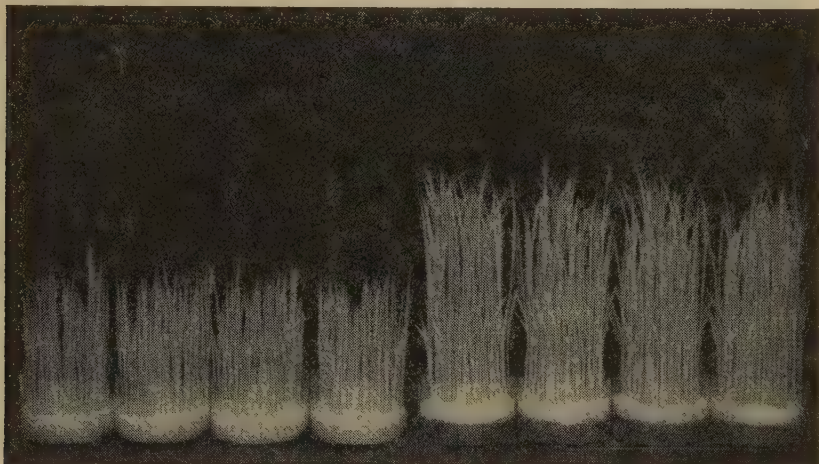


Fig. 2.—Twenty-day-old rice seedlings showing check at left and soil test at right. (Photographed by the Photographic Division, College of Agriculture.)

The seedlings were harvested after 20 days of growth, when it was assumed that the seedlings had assimilated all the nutrients in the soil.

Chemical analyses. The amount of P_2O_5 extracted from the soil ranged from 2.31 to 7.73 mg. per 100 grams of soil (table 1). The Neubauer values varied from zero to 3.97 mg. The values reported by Toh and Galvez⁴ ranged from 1.20 to 2.27 mg. for Elon-elon and from 1.49 to 1.90 mg. for Inintiw. The difference between the results of the Neubauer test and the rapid microchemical test is highly significant. It appears that the microchemical soil test gave more of the available phosphorus in the soil than the Neubauer method. The soil used in this experiment is a sandy clay loam which responds to fertilizer treatment, as shown by the increase in yield after fertilization. This increase cannot be entirely attributed to the phosphorus added since three elements are involved, but it is safe to say that the soil is not very deficient in phosphorus as it has been receiving fertilizer every planting season.

⁴ Toh Jin Siong and Nicolas L. Galvez, *Philippine Agriculturist*, 31, 118 (1947).



Fig. 3.—Newly harvested rice seedlings (check at left and treated at right) showing differences in height and root development. (*Photographed by the Photographic Division, College of Agriculture.*)

From the data obtained the Neubauer test using rice appears to have no value as indicator of phosphorus needs of soil. It may need considerable modifications before it can be applied to the soil type studied under Los Baños conditions. Therefore, the Neubauer test using rice cannot satisfactorily substitute for the rapid chemical soil test of Wolf and Ichisaka for phosphorus determination on the soil type investigated.

The quantity of K_2O extracted from the soil ranged from 16.42 to 29.88 mg., and that absorbed by the rice seedlings ranged from

25.36 to 41.70 mg. (table 1). The values reported by Toh and Galvez were from 0.75 to 1.10 mg. for Elon-elon and 0.68 to 1.00 mg. for Inintiw. The differences between these values and the results obtained in the present work are probably due to type of soil and variety of rice. The soil type which Toh and Galvez used was clay loam, which is heavier than the sandy clay loam employed in this experiment, and, according to Galvez,⁵ the amount of potash absorbed by rice seedlings increases as the texture of the soil becomes light. The data in table 1 show that in all the soil composites the Neubauer test gave higher values than the microchemical soil test. However, the Neubauer test is rather tedious and time-consuming.

For purposes of comparison, two methods, the perchloric acid and rapid chemical tests, were employed in determining the amount of potash extracted from the seedlings. The results of the two methods compare favorably (table 2).

There was no significant difference among the soil composites with regard to phosphoric acid and potash contents, indicating that the 15 paddies sampled contained the same amounts.

CONCLUSION

From this experiment, it is evident that the seedling test of Neubauer using rice as indicator and the rapid chemical soil tests are very different from each other. A biological test as an index of nutrient availability is subject to some physiological limitations. The assimilation of phosphorus and potassium by the seedlings is no doubt influenced by the presence of other cations, such as calcium and magnesium, and by other growth factors, such as temperature and aeration. It would be worthwhile making further studies of the Neubauer test under local conditions, with emphasis on these factors.

⁵ N. L. Galvez, *Journal für Landwirtschaft*, 82, 257 (1934).

TABLE 1.—*Available phosphoric acid and potash in the soil expressed in milligrams per hundred grams of soil as determined by two methods*

COMPOSITE ^a	PHOSPHORIC ACID		POTASH	
	Neubauer test	Microchemical soil test	Neubauer test	Microchemical soil test
1	1.16	4.46	33.65	19.71
2	3.38	5.34	35.35	19.82
3	1.07	3.52	31.87	21.55
4	0.28	3.70	28.91	17.11
5	1.00	4.15	37.98	18.80
6	2.41	3.42	38.18	24.13
7	1.08	3.62	34.32	21.04
8	0.43	4.54	32.78	19.83
9	0.58	4.02	33.82	22.51
10	2.03	3.82	39.94	20.26
11	2.34	3.02	36.98	20.51
12	0.80	4.14	34.98	18.27
13	1.94	6.48	32.50	27.67
14	0.38	6.17	32.16	26.90
15	0.44	7.24	33.30	26.66
Mean	1.29	4.51	34.44	21.65

^a Quadruplicated.L.S.M.D., P_2O_5 = 0.91 mg./100 g.
K₂O = 2.54 mg./100 g.TABLE 2.—*Root soluble potash in milligrams extracted from the seedlings by two methods*

COMPOSITE ^a	Perchloric acid test	Microchemical soil test
1	33.65	31.10
2	35.35	36.97
3	31.87	34.31
4	28.91	29.32
5	37.98	37.12
6	38.18	23.39
7	34.32	33.64
8	32.78	28.31
9	33.82	32.60
10	39.94	39.88
11	36.98	32.87
12	34.98	35.42
13	32.50	24.22
14	32.16	24.38
15	33.30	34.50
Mean	34.44	31.87

^a Quadruplicated.

L.S.M.D. = 2.58 mg., suggestive

THE DIGESTIBILITY OF *CENTROSEMA PUBESCENS* AND *PUERARIA JAVANICA*¹

BENJAMIN REYES

Seven-day feeding experiments showed that *Pueraria javanica* Benth. was more digestible than *Centrosema pubescens* Benth. in N-free extract for sheep and in organic matter and crude fiber for goats. *Centrosema* had more digestible protein than tropical kudzu, although kudzu had more total digestible nutrients. The results indicated that sheep could digest organic matter and crude fiber in *Centrosema* and organic matter, crude fiber, ether, and N-free extract in kudzu better than goats. These differences might have been due to age or species.

Centrosema pubescens and *Pueraria javanica*, or tropical kudzu, are leguminous, vinelike forages which show promise as high-protein livestock feeds in the Philippines. *Centrosema*, introduced here in 1938 by Dr. V. Villegas, is widely used as a cover crop and has been found to be well-adapted to local conditions. Tropical kudzu, on the other hand, thrives well in cultivated fields, is palatable, and has a high yield. The present study was conducted in the Department of Animal Husbandry from November, 1953, to May, 1954, to determine the digestibility of tropical kudzu and *Centrosema* by sheep and goats.

MATERIALS AND METHODS

Four grade Shropshire sheep and four native goats were used. Male animals were chosen to facilitate the separation of the feces from the urine. The sheep were a little over 3 years and 4 months, and the goats, a few days over 1 year and 10 months, at the start of the experiment. Near the middle of the experiment a goat died of toxemia and so another pair of approximately the same age was substituted.

The animals, paired according to ages, were placed in metabolism cages. They were subjected to a 10-day preliminary feeding period on constant intake before the 7-day fecal collection period was started. Their initial and final weights were taken.

Four feeding trials were done. During each trial, the same amount of *Centrosema* or tropical kudzu alone, besides salt and water, was given the animals morning and afternoon.

Samples of the feed given during each feeding were collected, dried under infrared lamps, and composited for analysis in the Cen-

¹ Central Experiment Station Contribution No. 1825. Thesis presented for graduation, October, 1954, with the degree of Bachelor of Science in Agriculture from the College of Agriculture. Prepared in the Department of Animal Husbandry under the direction of Dr. John K. Loosli and Dr. Valente Villegas.

tral Experiment Station Analytical Laboratory according to A.O.A.C. methods.²

The average proximate analyses of the two forages (fresh basis) were as follows:

CONSTITUENT	CENTROSEMA per cent	TROPICAL KUDZU per cent
Moisture	75.77	77.33
Crude protein	5.01	3.65
Ether extract	1.17	0.78
Crude fiber	8.26	8.40
N-free extract	7.78	7.41
Ash	2.00	2.35
Dry matter	24.22	22.59

The feces were collected each day at the same hour for seven consecutive days and weighed. One-tenth samples were air-dried under an infrared lamp and weighed before they were bottled in tightly closed Mason jars. The orts were gathered each day for their fresh and air-dry weights.

The composited feeds, refused feeds, and feces collected in one feeding trial were thoroughly mixed and quartered for sampling. Composite aliquoted samples were analyzed for moisture, crude protein, crude fiber, ether extract, ash, and nitrogen-free extract. The average digestion coefficients were determined in the manner explained by Maynard,³ and the digestive nutrients of the feeds, by Morrison's methods.⁴

RESULTS AND DISCUSSION

The differences in the digestion coefficients obtained for sheep for the two feeds were significant only for N-free extract (table 1a). For goats kudzu was apparently more digestible in organic matter and crude fiber than Centrosema (table 1b). No significant differences were found in the digestibilities of the crude protein, ether, and N-free extract of the two feeds.

Sheep were found to digest the organic matter and crude fiber of Centrosema better than goats (table 2a). The differences in the digestion coefficients of the other nutrients for the two species were not significant.

Sheep had higher mean digestion coefficients than goats for all the nutrients of kudzu studied (table 2b). The differences in the digestion coefficients were all significant, except for crude fiber.

That the sheep could digest the organic matter and crude fiber of Centrosema and the organic matter, crude protein, ether, and

² Assoc. Off. Agric. Chem., "Official Methods of Analysis of the Association of Official Agricultural Chemists," A.O.A.C., Washington, D.C., 1950, 910 pp.

³ L. A. Maynard, "Animal Nutrition," McGraw-Hill Book Co., Inc., New York, 1951, 474 pp.

⁴ F. B. Morrison, "Feeds and Feeding," The Morrison Publishing Co., Ithaca, New York, 1951, 1050 pp.

N-free extract of tropical kudzu better than goats might have been due to the difference in species or in age.

Centrosema had more digestible protein than tropical kudzu (table 3). Although the average digestion coefficients of sheep and goats were higher for kudzu, a high proximate chemical analysis value gave Centrosema more digestible protein. Although kudzu and Centrosema had almost the same amount of crude fiber, tropical kudzu turned out to have more digestible crude fiber than Centrosema. Tropical kudzu also had less fat and more digestible N-free extract than Centrosema. In total digestible nutrients, tropical kudzu had a slightly higher value than Centrosema.

TABLE 1a.—Comparative digestibility of *Centrosema* and tropical kudzu by sheep (paired method)

ITEM	ORGANIC MATTER	CRUDE PROTEIN	ETHER EXTRACT	CRUDE FIBER	N-FREE EXTRACT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Average digestion coefficient for Centrosema	54.02	64.3	57.0	43.4	59.6
Average digestion coefficient for kudzu	61.4	77.2	50.4	50.4	66.8
Mean difference (MD)	7.38	12.9	6.6	7.0	7.2
SE of MD	2.85	6.16	6.65	2.35	0.59
<i>t</i> -Value	2.59	2.10	0.99	2.95 ^a	12.34
Probability	.05	.05	.05	.05	.05

^a Suggestive.

$$\text{tab. } t = .01,3 = 3.182$$

TABLE 1b.—Comparative digestibility of *Centrosema* and tropical kudzu by goats (paired method)

ITEM	ORGANIC MATTER	CRUDE PROTEIN	ETHER EXTRACT	CRUDE FIBER	N-FREE EXTRACT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Average digestion coefficient for Centrosema	46.2	62.1	50.0	27.2	51.6
Average digestion coefficient for kudzu	57.0	71.2	48.7	48.0	61.6
Mean difference (MD)	10.8	9.1	1.3	20.8	10.00
SE of MD	1.92	4.15	5.18	4.35	3.02
<i>t</i> -Value	5.62	2.20 ^a	.25	4.78	2.00
Probability	.05	.05	.05	.05	.05

^a Suggestive.

$$\text{tab. } t = .05,5 = 2.571$$

TABLE 2a.—*Digestion coefficients of Centrosema for sheep and goats (pooled method)*

ITEM	ORGANIC MATTER	CRUDE PROTEIN	ETHER EXTRACT	CRUDE FIBER	N-FREE EXTRACT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Average digestion coefficient for sheep	54.02	64.28	57.00	43.45	59.58
Average digestion coefficient for goats	46.18	62.10	50.03	27.22	51.57
Mean difference (MD)	7.84	2.18	6.97	16.23	8.01
SE of MD	2.23	3.28	4.25	2.57	3.69
<i>t</i> -Value	3.52	.66	1.63	6.32	2.17
Probability	.05	.05	.05	.05	.05

tab. *t* = .05,8 = 2.306TABLE 2b.—*Digestion coefficients of kudzu for sheep and goats (pooled method)*

ITEM	ORGANIC MATTER	CRUDE PROTEIN	ETHER EXTRACT	CRUDE FIBER	N-FREE EXTRACT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Average digestion coefficient for sheep	61.40	77.2	50.38	50.44	66.85
Average digestion coefficient for goats	56.97	71.25	48.68	48.03	61.62
Mean difference (MD)	4.43	5.95	1.7	2.01	5.23
SE of MD	.91	1.39	.39	2.45	1.11
<i>t</i> -Value	4.87	4.28	4.36	0.82	4.71
Probability	.05	.05	.05	.05	.05

tab. *t* = .05,8 = 2.306TABLE 3.—*Average digestible nutrients of Centrosema and tropical kudzu ^a*

FEED	PROTEIN	FAT	FIBER	N.F.E.	TOTAL DIGESTIBLE NUTRIENTS	NUTRITIVE RATIO
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Centrosema	3.16	1.41	2.92	4.32	11.81	1:2.74
Tropical kudzu ..	2.70	0.87	4.13	4.75	12.45	1:3.61

^a Based on sample means.

THE DIGESTIBILITY OF ANDROPOGON NODOSUS¹

MANUEL V. SOLIVEN

The young stage of *Andropogon nodosus* (Willem.) Nash. had a significantly higher digestibility for crude protein than the medium stage and the late stage. The digestibility of crude fiber was highest in the early stage and lowest in the late stage. Similar results were observed with mean values of nitrogen-free extract. The digestion coefficients of organic matter in the early stage and the medium stage varied insignificantly. They were considerably higher than the digestion coefficient at the mature stage. Ether extract tended to increase in digestibility from the early stage to the medium stage and the late stage.

Andropogon nodosus, together with Para grass (*Panicum purpurascens* Raddi), is recommended for the improvement of pastures. It is a semi-erect, fine-textured, perennial grass attaining the height of 1½ meters at maturity. This palatable forage grass grows well and multiplies from runners and seeds, producing 20 to 30 stalks to a hill. The stalks are slim but tough.

The digestibility and the proper stage of maturity at which this grass should be fed have not previously been studied. Accordingly, the digestibility of *A. nodosus* at the early, medium, and late stages of maturity was investigated from August, 1953, to September, 1954, in the Department of Animal Husbandry.

MATERIALS AND METHODS

Five wethers were used in each experiment: Herds No. 188, 190, 191, 194, and 197. Sheep were selected for the study because they are easy to handle and their feed requirements are small. Providing a uniform supply of pure stands of the forage crop was consequently less expensive. All the five sheep were used in the late-maturity stage and medium-maturity stage experiments. But before the experiment on the early-maturity stage could be begun, animals No. 188 and 190 died of enterotoxemia. They were replaced by Herd No. 226 and Herd No. 250.

In digestion stalls the animals were fed Para grass and a concentrate mixture for approximately a month to accustom them to confinement and to being handled. They were then fed *A. nodosus* for a preliminary period of five to ten days to standardize the feed intakes and to allow the residue of former feeds to pass out of the digestive tract. After this, fecal collections were started. During the collection period, the same amount of *A. nodosus* was given each day. Salt and clean, fresh water were made available to the animals every day. Collections were made for eight days for the late stage,

¹ Central Experiment Station Contribution No. 1826. Thesis presented for graduation in October, 1954, with the degree of Bachelor of Science in Agriculture. Prepared in the Department of Animal Husbandry under the direction of Dr. John K. Loosli.

seven days for the medium stage, and six and seven days for the first and second trials of the early stage experiment. The feces were weighed at the same hour each day and carefully mixed. Ten per cent of the mixture was dried under infrared lamps, pulverized, composited, and, to prevent spoilage, stored in sealed Mason jars until chemically analyzed.

At each feeding (8:00 a.m. and 3:00 p.m.) during the collection period, a sample of the forage was dried for determination of the moisture content, and the samples were composited and saved for analysis. The same procedure was followed with any orts that were obtained. In all studies the feed rations were limited to minimize feed refusals. The orts occasionally obtained were analyzed and considered in the calculations.

The animals were weighed before and after each feeding experiment. Four separate studies were carried out involving a total of 15 trials with seven different sheep.

A. nodosus was harvested for the early stage experiment four weeks after the grass had been cut. It was cut gradually, a portion of the field every day, to assure uniform age of the forage throughout the feeding experiment. The grass was cut close to the ground and all cuttings were raked out. The grass for the early stage experiment had an average height of 46.2 centimeters; for the medium stage, 93.2 centimeters; and for the late stage, 122.4 centimeters. The late stage was differentiated from the medium stage by the presence of inflorescence in the major portion of the field.

Fecal examinations revealed mild to moderate infestations of stomach worms (*Haemonchus contortus*), stomach flukes (*Cotylphoron cotylphorum*), and coccidia oocysts.² The animals were treated with 5 to 20 grams of phenothiazine according to body weight.

Proximate chemical analyses according to the methods of A.O.A.C. were made at the close of each trial on composite, aliquoted samples of feeds, orts, and feces.³ The coefficients of digestibility were determined in the manner explained by Maynard.⁴

RESULTS AND DISCUSSION

During each study the animals generally lost weight, though none lost more than 0.8 of a kilogram, indicating that they were fed their approximate maintenance requirements (table 1).

Table 3 summarizes the average daily air-dry matter of the grass fed, orts, intake, feces, and amount digested and the percentage digested by each animal of each stage of the grass. It will be noted

² Dr. J. D. Generoso of the Bureau of Animal Industry, Manila, identified the intestinal parasites.

³ Analyses were made at the Central Experiment Station Chemical Laboratory.

⁴ Leonard A. Maynard, "Animal Nutrition," McGraw-Hill Book Co., Inc., New York, Toronto, London, 1951, 474 pp.

that there was a large amount of orts in the late stage grass, less in the medium stage, and much less in the early stage. This may have been partly due to the proportions of the leaves and the stems, which are tough and unpalatable. The early stage grass had 60.6 per cent stems by weight, air-dry basis; the medium stage grass had 69.9 per cent stems; and the late stage grass had 73.2 per cent stems. The digestibility of air-dry matter was influenced by the stage of maturity. The early stage grass had an average digestibility of 61.6 per cent; the medium stage grass, 59.1 per cent; and the late stage grass, 38.2 per cent.

The composition of the forage studied at the three different stages of maturity is shown in table 2. Two separate studies were made of the early stage because the yield of grass was low at this stage.

The chemical analysis for organic matter showed that there was more organic matter in the early stage than in the medium stage (table 2). Then there was an increase in the amount of organic matter from the medium stage to the late stage. The digestibility of organic matter tended to be greatest in the early stage, less in the medium stage, and least in the late stage (table 4a). The analysis of variance between stages was significant. Computation for L.S.M.D. identified the means of the early stage and the medium stage as coming from one population, which varied significantly from the late stage. Analysis of variance for animals indicated a significant difference between animal No. 190 (226)⁵ and No. 191 and No. 197. This difference might have been due partly to parasites found in their intestinal tracts and partly to their individual physiological variations. However, the parasites in the intestinal tracts of the animals might not have caused too great a difference as was shown by Loosli *et al.*⁶

The digestibility and chemical analysis values of crude protein coincided in their tendency to decrease considerably from the early stage to the medium stage and to rise slightly in the late stage (tables 2 and 4b). The unexpected rise in the protein content of the grass in the analysis of the medium and late stages might have been due to the presence of inflorescence in the late stage. Calculation of the L.S.M.D. of the mean digestion coefficients of the medium and late stages showed them to be of the same population apart from the mean of the early stage. Sheep No. 191 in the medium stage experiment had — 8.8 value for crude protein (table 4b), indicating that it had more protein in the excreta than it was taking in. This may be accounted for by bacterial action or the excretion of metabolic protein in the intestinal tract.⁷

⁵ Animal No. 226 replaced No. 190, and No. 250 replaced No. 188 in the early stage experiment.

⁶ J. K. Loosli, V. Villegas, G. V. Garcia, and L. A. Ynalvez, *Philippine Agriculturist*, 37, 520 (1954).

⁷ Frank B. Morrison, "Feeds and Feeding," Morrison Publishing Co., Ithaca, New York, 1951, 1207 pp.

The percentage of fat appeared to increase from the early stage to the medium stage and to the late stage (table 2). The same trend was noted in the digestion coefficients which had highly significant variations between stages (table 4c). The variation of the means of the animals for ether extract was significant to the .05 level.

Crude fiber decreased in digestibility from the early stage to the medium stage and from thence to the late stage (table 4d). Analysis of variance showed high significance between all stages. The means for crude fiber of sheep No. 188 (250), No. 190 (226), and No. 191 belonged to the same population. The means of sheep No. 194 and No. 197 belonged to another population.

Nitrogen-free extract showed highly significant differences in mean values of digestion coefficients (table 4e). In the chemical analysis, the medium stage showed the least amount of nitrogen-free extract (table 2).

TABLE 1.—*Weights of animals before and after each experiment*

STAGE OF MATURITY	HERD NO.	INITIAL WEIGHT	DATE OF WEIGHING	FINAL WEIGHT	DATE OF WEIGHING
Early	191	17.8	August 24, 1954	17.4	August 31, 1954
	194	13.7	August 14, 1954	12.9	August 21, 1954
	197	15.0	August 14, 1954	14.6	August 21, 1954
	226	10.8	August 14, 1954	10.8	August 21, 1954
	250	13.3	August 24, 1954	12.8	August 31, 1954
Medium	188	10.9	November 23, 1953	10.9	December 1, 1953
	190	10.5	November 23, 1953	10.6	December 1, 1953
	191	16.6	November 23, 1953	16.4	December 1, 1953
	194	9.3	November 23, 1953	8.9	December 1, 1953
	197	10.7	November 23, 1953	10.4	December 1, 1953
Late	188	11.0	October 7, 1953	10.4	October 16, 1953
	190	10.6	October 7, 1953	10.4	October 16, 1953
	191	16.7	October 7, 1953	16.0	October 16, 1953
	194	9.3	October 7, 1953	9.0	October 16, 1953
	197	10.7	October 7, 1953	9.9	October 16, 1953

TABLE 2.—*Average proximate analysis of soilage at three different stages*

STAGE OF MATURITY	ORGANIC MATTER	CRUDE PROTEIN	ETHER EXTRACT	CRUDE FIBER	N-FREE EXTRACT	ASH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Early	84.15	9.08	2.40	33.08	39.59	13.34
Early	82.92	5.61	2.88	33.62	40.81	11.88
Medium	73.29	4.23	3.38	33.55	32.13	10.39
Late	81.38	4.64	6.27	37.00	33.47	9.69

TABLE 3.—Average daily air-dry matter of soilage, orts, intake, feces, and amount digested and the percentage digested

ITEM	SHEEP NO. 250 ^a	SHEEP NO. 226 ^b	SHEEP NO. 191	SHEEP NO. 194	SHEEP NO. 197
<i>Early stage</i>					
Soilage	179.2	387.2	384.1	484.0	677.6
Orts	5.9	11.1	4.2	6.8	6.7
Intake	173.3	376.1	378.9	477.2	670.9
Feces	84.4	134.6	193.4	147.2	172.5
Amount digested	88.9	241.5	186.5	330.0	498.4
Percentage digested .	51.3	64.2	49.1	69.2	74.3
<i>Medium stage</i>					
Soilage	332.8	332.8	457.6	249.6	249.6
Orts	76.0	18.3	65.0	5.8	15.7
Intake	256.8	314.5	392.6	243.8	233.9
Feces	109.8	129.5	165.9	99.3	87.2
Amount digested	147.0	185.0	226.7	144.5	145.7
Percentage digested .	57.2	58.8	57.7	59.3	62.3
<i>Late stage</i>					
Soilage	294.8	308.1	497.7	248.8	284.4
Orts	111.6	111.2	161.6	80.0	101.9
Intake	183.2	196.9	336.1	168.8	182.5
Feces	108.3	139.0	223.2	99.7	98.0
Amount digested	74.9	57.9	112.9	69.1	84.5
Percentage digested .	40.9	29.4	33.6	40.9	46.3

^a Replaced by Sheep No. 188 in the studies on the medium and late stages.^b Replaced by Sheep No. 190 in the studies on the medium and late stages.TABLE 4a.—Digestion coefficients for organic matter of *A. nodosus* at three stages of maturity

HERD NUMBER	EARLY STAGE	MEDIUM STAGE	LATE STAGE	TOTAL	MEAN
188 (250)	58.0	59.7	41.8	159.5	53.2
190 (226)	67.6	57.9	30.4	155.9	52.0
191	55.3	54.2	36.8	146.3	48.8
194	72.5	60.2	41.4	174.1	58.0
197	78.3	61.6	46.7	186.6	62.2
Total	331.7	293.6	197.1	822.4	
Mean	66.3	58.7	39.4		

Analysis of variance

S.V.	D.F.	S.S.	M.S.	F.	TAB. F	
Stage (S) ...	2	1,925.40	962.70	34.93	.05 4.46	.01 8.65
Animal (A) .	4	336.92	84.23	30.56	3.84	7.01
S × A	8	220.45	27.56			
Total	14	2,482.77				

L.S.M.D. (S) = 7.66

L.S.M.D. (A) = 9.87

TABLE 4b.—*Digestion coefficients for crude protein of A. nodosus at three stages of maturity*

HERD NUMBER	EARLY STAGE	MEDIUM STAGE	LATE STAGE	TOTAL	MEAN
188 (250)	50.3	4.7	15.5	70.5	23.5
190 (226)	38.8	13.0	13.9	65.7	21.9
191	48.6	—8.8	17.5	57.3	19.1
194	46.3	25.7	26.0	98.0	32.7
197	57.7	23.3	25.7	106.7	35.6
Total	241.7	57.9	98.6	398.2	
Mean	48.3	11.6	19.7		

Analysis of variance

S.V.	D.F.	S.S.	M.S.	F.	TAB. F	
Stage (S) ..	2	3,727.77	1,863.88	29.557	.05	.01
Animal (A) .	4	615.43	153.86	2.44	4.46	8.65
S × A	8	504.50	63.06		3.84	7.01
Total	14	4,847.70				

L.S.M.D. (S) = 11.58

TABLE 4c.—*Digestion coefficients for ether extract of A. nodosus at three stages of maturity*

HERD NUMBER	EARLY STAGE	MEDIUM STAGE	LATE STAGE	TOTAL	MEAN
188 (250)	50.0	61.1	60.8	171.9	57.3
190 (226)	50.9	61.0	66.8	178.7	59.6
191	51.6	70.5	74.3	196.4	65.5
194	58.0	70.7	78.0	206.7	68.9
197	60.3	61.5	80.6	202.4	67.5
Total	270.8	324.8	360.5	956.1	
Mean	54.2	65.0	72.1		

Analysis of variance

S.V.	D.F.	S.S.	M.S.	F.	TAB. F	
Stage (S) ..	2	815.78	407.89	21.239	.05	.01
Animal (A) .	4	307.16	76.79	3.998	4.46	8.65
S × A	8	153.64	19.21		3.84	7.01
Total	14	1,276.58				

L.S.M.D. (S) = 6.39

L.S.M.D. (A) = 8.23

TABLE 4d.—*Digestion coefficients for crude fiber of A. nodosus at three stages of maturity*

HERD NUMBER	EARLY STAGE	MEDIUM STAGE	LATE STAGE	TOTAL	MEAN
188 (250)	66.2	66.8	47.5	180.5	60.2
190 (226)	76.4	66.5	35.9	178.8	59.6
191	63.0	62.3	33.4	158.7	52.9
194	80.4	71.6	54.0	206.0	68.7
197	85.6	71.4	52.2	209.2	69.7
Total	371.6	338.6	223.0	933.2	
Mean	74.3	67.8	44.6		

Analysis of variance

S.V.	D.F.	S.S.	M.S.	F.	TAB. F	
Stage (S) ..	2	2,435.62	1,217.81	51.083	.05	.01
Animal (A) .	4	587.86	146.97	6.165	4.46	8.65
S × A	8	190.72	23.84		3.84	7.01
Total	14	3,214.20				

L.S.M.D. (S) = 6.39

L.S.M.D. (A) = 8.23

TABLE 4e.—*Digestion coefficients for nitrogen-free extract of A. nodosus at three stages of maturity*

HERD NUMBER	EARLY STAGE	MEDIUM STAGE	LATE STAGE	TOTAL	MEAN
188 (250)	53.6	61.1	35.7	150.4	50.1
190 (226)	65.6	54.2	17.2	137.0	45.7
191	50.6	53.5	33.5	137.6	45.9
194	70.8	52.0	21.3	144.1	48.0
197	76.3	56.8	35.5	168.6	56.2
Total	316.9	277.6	143.2	737.7	
Mean	63.4	55.5	28.6		

Analysis of variance

S.V.	D.F.	S.S.	M.S.	F.	TAB. F	
Stage (S) ..	2	3,318.63	1,659.315	21.491	.05	.01
Animal (A) .	4	224.47	56.118	0.727	4.46	8.65
S × A	8	617.68	77.21		3.84	7.01
Total	14	4,160.78				

L.S.M.D. (S) = 5.56

TYPES OF LAYING HOUSES FOR THE FARM ¹

F. M. FRONDA AND A. C. CAMPOS

WITH FIVE TEXT FIGURES

Fifty weeks' study of a conventional type of poultry house, one open on all sides, and two partially walled ones showed that a greater proportion of the birds in the more open types of houses were in better physical condition than those in the closed type. Mortality did not seem to be distinctly related to the type of house used. The flock in the house where only the roosts were protected by a 35-centimeter wall on the north, west, and south sides laid the most eggs and yielded the highest profit.

In poultry production, housing layers is sometimes considered a necessary evil because the birds are forced to live under conditions unnatural to them. In many instances the cost of housing is the most expensive single item in a poultry enterprise. Farmers planning to raise poultry are, therefore, confronted with two problems: how to build laying houses with the comforts necessary to induce the birds to perform profitably, and how to build them with the least possible expense. To find solutions to these problems, this study was conducted in the Department of Animal Husbandry from October 1, 1953, to September 15, 1954.

MATERIALS AND METHODS

Four types of laying houses with the same size, roof type, exposure, and construction materials of flattened asphalt barrels and ipil-ipil posts were built (figs. 1 to 4). Each house measured 2.5 by 2.5 meters and was 1.3 meters high in front and 0.8 meter behind. The first house (lot I) was open on all sides; the second (lot II) had the roosts protected by a 35-centimeter wall on the north, west, and south sides leaving the bottom and top open; the third (lot III) had the sides all walled, with a 30-centimeter opening at the bottom; and the fourth (lot IV) was the conventional type with three sides completely walled from ground to roof. The fronts of all the houses were open. Every house had three rows of roosts running north to south, a community nest that could accomodate four birds at a time, a 1 1/3-meter range feeding trough, and a drinking bucket with stand. Each one was located in the center of a grassy yard, 13 by 16 meters, which provided fresh green grass throughout the year.

Thirty S. C. White Leghorn pullets that were just beginning to lay were placed in each house at the start of the experiment. These birds were picked from the general flock of the College and were randomly assigned to the four lots. The birds, except those of lot I,

¹ Central Experiment Station Contribution No. 1827. Progress report of research project No. 303 of the Department of Animal Husbandry.



Fig. 1.—Lot I laying house open on all sides.



Fig. 2.—Lot II laying house with the roosts protected by a 35-centimeter wall on the north, west, and south sides and the bottom, and top open.



Fig. 3.—Lot III laying house with the sides all walled and a 30-centimeter opening at the bottom.



Fig. 4.—Lot IV laying house with three sides completely walled from ground to roof.

were legbanded with colored celluloid rings for identification. All the lots had free access to the College all-mash laying ration made up of the following ingredients, all parts by weight:

Ground yellow corn	50
Fine rice bran	20
Copra meal	10
Fish meal	20
Ground shells and grit	<i>ad libitum</i>

The same care and management was given to the birds in the four lots. Except for the protection afforded by the houses, no extra precautions were taken to shield the birds against inclement weather. There was no shade in the yards. Culling was not done during the study. The birds that became sick were left to stay in the pen until they died.

Records of the initial and final weights of the birds, amount of feed consumed, number of birds that died, and daily egg production of each lot were kept.

RESULTS AND DISCUSSION

Body weight. The pullets in the four lots had practically the same average weights at the start of the study, the range being only 30 grams (table 1). Having been randomly picked from one stock which hatched at about the same time, these pullets had an even chance to gain or lose in weight if exposed to the same environment and given the same feed and care. The difference in housing, however, appeared to have caused a slight disparity in their final weights. The birds in lot I registered the highest average final weight, followed closely by those in lot III. When the flock was inspected at the end of the study for possible use in the breeding pens, a greater proportion of the birds in lots I, II, and III were in a better condition to go through another year than those in lot IV.

Mortality. Unlike the weight of the birds, the rate of mortality did not seem to have been influenced by the house type, corroborating the finding of Christopher and King.² Lot III had the highest mortality and lot IV had the lowest (table 1). The total mortality in the four lots was only less than 20 per cent, which may be considered well within the normal mortality expectations. These results demonstrate that laying hens given good feed, a clean grassy pasture, and reasonable protection against inclement weather will survive under ordinary farm conditions.

Egg production. During the 50-week study, the birds in lot II laid the highest number of eggs, 1,246 more than the least productive group, lot IV (table 1). In terms of average production of a bird, lot II had the most productive birds and lot IV the least. When the total number of eggs produced and percentage mortality in lots I

² R. C. Christopher and D. F. King, *Alabama Polytechnic Inst. Agric. Exper. Sta. Cir.*, 88 (1943).

and III were analyzed, each bird in lot I appeared to have produced more eggs than those in lot III when more eggs and fewer birds were credited to lot III than to lot I. This apparent discrepancy is attributed to the fact that of the 26.7 per cent mortality (8 deaths) in lot III, 6.66 per cent (2 deaths) occurred during the last 15 days of the test so that during the period of the study there were more living birds in lot III (average of 25.7 birds) than in lot I (average of 24.5 birds).

Soon after the start of the test, the egg production of the birds in the conventional type house (lot IV) tended to lag behind those of the other lots (fig. 5). On the other hand, the egg production curves of lots I, II, and III rose sharply from the beginning up to the peak month, February, then gradually dropped until the close of the experiment. Unlike lots I and III, however, lot II registered a markedly higher percentage of monthly production from February to August. The February production of lot II was almost 85 per cent, whereas those of lots I and III were below 70 per cent. After February, the production curve of lot II remained above, and those of lots I and III crisscrossed each other.

It is interesting to note that notwithstanding the type of house, the production curve of all the lots followed the same pattern. Christopher and King³ found that hens kept in an expensive house produced only 8 per cent more eggs than those without a house and only 3 per cent more than those in a conventional, three-sided, inexpensive house. Another point worth noting is the fairly high average production of the pullets in all the lots except lot IV. For a backyard flock with such simple houses and whose surroundings became filthy during the rainy months, the pullets used in this study, except those in lot IV, had a remarkable production performance.

Amount and cost of feed consumed to produce a dozen eggs. To gauge the feed utilization efficiency of the birds kept in the four types of houses, the amounts of feed consumed to produce a dozen eggs were computed. Lot II needed the least to produce a dozen eggs, and lot IV needed the most (table 1). The ration given to the birds cost ₱0.26 a kilogram so that lot II required ₱0.49 worth of feed to produce a dozen eggs; lot IV, ₱0.59; lot I, ₱0.53; and lot III, ₱0.55. Judging from these figures, lot II was the most efficient converter of feed, followed by lots I, III, and IV in descending order.

Returns above costs. Assuming that feed costs comprise 60 per cent of the total cost of production⁴ and that the housing costs of the four lots were the same, lot II needed an over-all expense of ₱0.82 to produce a dozen eggs; lot IV, ₱0.97; lot I, ₱0.88; and lot III, ₱0.91. At ₱0.12 an egg, lot II would have netted ₱0.62 for every dozen eggs produced, or ₱276.29 for all eggs laid. The corresponding figures for lot IV would have been ₱0.47 for every dozen eggs

³ *Ibid.*

⁴ F. M. Fronda and P. S. Paje, *Philippine Agriculturist*, 19, 337 (1930).

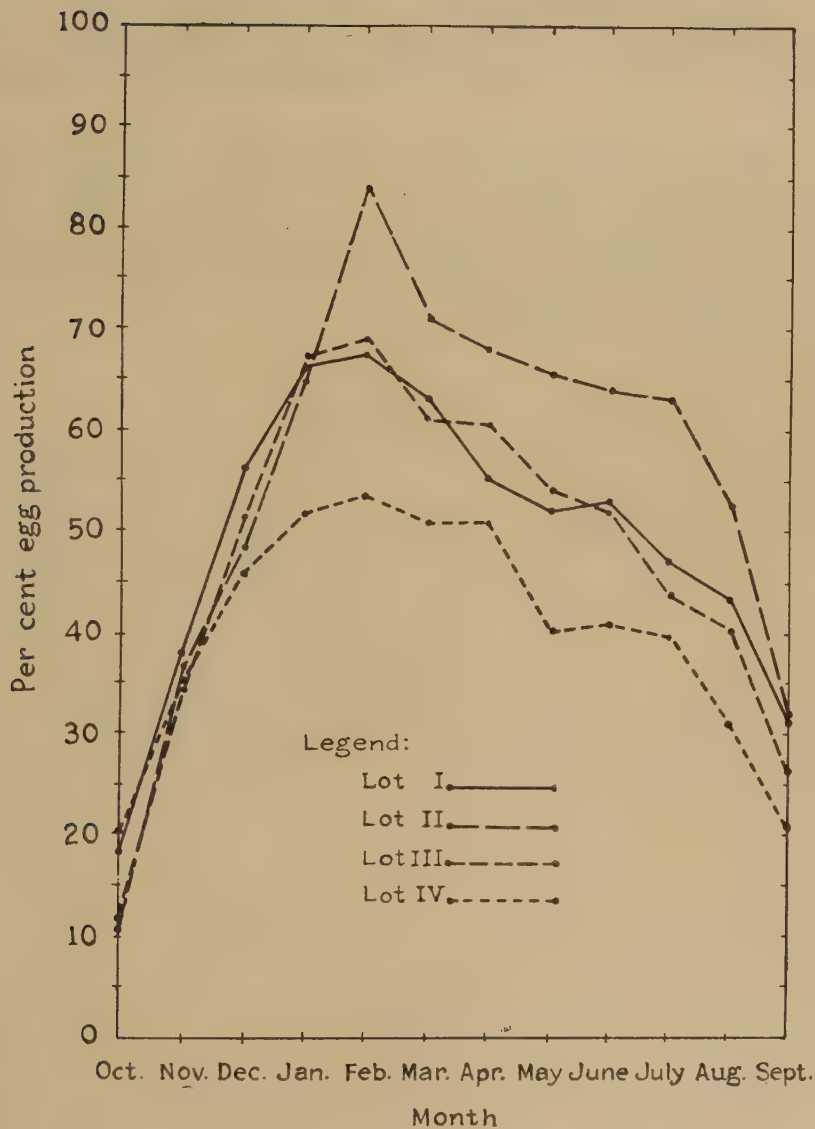


Fig. 5.—Egg production in the four lots.

or ₱158.92 for all the eggs produced; for lot I, ₱0.56 for every dozen or ₱199.65 for all; and for lot III, ₱0.53 for every dozen or ₱191.58 for all eggs laid.

From the preceding figures it will be seen that the net profit derived during the year from lot II birds, the best producers, was about $1\frac{2}{3}$ times as much as that from the poorest group, lot IV, and approximately $1\frac{1}{3}$ times more than those from the other two lots.

Under the conditions of this study, the most profitable type of house to use for a small farm flock is apparently one that has 35-centimeter walling on the north, west, and south sides opposite the roosts to protect the birds from drafts during inclement weather. In contrast, Christopher and King⁵ computed lower feed and housing costs for every dozen eggs from the three-sided type of house than from any other type studied.

⁵ *Ibid.*

TABLE 1.—*Summary table on types of laying houses for the farm*

ITEM	LOT I (Open on all sides)	LOT II (Thirty-five cm. walling)	LOT III (Open at the bottom)	LOT IV (Closed on three sides)
Average weight at the start, kilograms	1.22	1.20	1.19	1.20
Average weight at the close, kilograms	1.44	1.36	1.42	1.30
Number of eggs produced	4,263	5,348	4,372	4,102
Egg production of a bird, num- ber	173.4	194.0	168.9	142.7
Egg production of a bird, per cent	49.7	55.6	48.4	40.9
Feed consumed by a bird, kilo- grams	29.26	30.56	31.80	26.45
Feed needed to produce a dozen eggs, kilograms	2.03	1.89	2.11	2.25
Mortality, per cent	23.3	13.3	26.7	10.0
Selection at the close of the test, per cent	69.1	53.8	59.1	44.4

COLLEGE AND ALUMNI NOTES

One hundred eleven graduates of the College of Agriculture were among the 1400 who were awarded their diplomas by Dr. Vidal A. Tan at the general commencement exercises held in Diliman on April 15. President Ramon Magsaysay delivered the commencement address.

Rodolfo Mendiola Ela, *magna cum laude*, was the only aggie to graduate with honors.

Class '55 includes the first graduates of the home technology course.

Graduates with the degree of Bachelor of Science in Agriculture were Antonio S. Achacoso, Eusebio V. Alava, Francisco G. Alcasid, Romeo A. Alicbusan, Valentino C. Alipio, Martin B. Alvarez, Reynaldo A. Alviar, Gabriel D. Amigo, Alberto A. Arellon, Benjamin L. Arenas, Catalino G. Aurelio Jr., Bonifacio A. Azucena Jr., Pedro L. Bagoyo, Teodoro B. Baguilat, Alvaro N. Balcita, Jovencio S. Balcita, Rodolfo C. Banogon, Teofilo S. Batin, Fernando A. Bernardo, Hermenegildo B. Bernabe, Vicente C. Bruce, Jesus B. Caingcoy, Feliciano B. Calora, Virgilio R. Carangal, Jose C. Carreon, Demetrio M. Castillo, Mauro V. Catalan, Arsenio E. Corpuz;

Felicitas L. Cortes, Leonardo C. Cosico, Santiago H. Dacanay, Mercedes D. Delfinado, Vicente C. Dosado, Rodolfo M. Ela, Ofelio R. Exconde, Rollo G. de la Fuente, Luz C. Esguerra-Gabertan, Silvestre G. Gabot, David D. Gorrez, Francisco D. Gorrez, Leopoldo P. de Guzman, Perfecto P. Hebron, Frederick R. Ick, Hermogenes S. Lanao, Archimides L. Laurel, Benjamin M. Legaspi, Cesar P. Librodo, Roberto T. Lim, Vicente O. Lumawag, Antonio C. Lumbres, Juan C. Lusanea, Urbano M. Macasaet, Isabel O. Malabanan, Cezar Mamaril, Feliciano C. Manuel, Gil T. Mansa;

Simplicio R. Manzano, Fortunato S. Melodia, Severino O. Memita, Eleuterio M. Mendoza, Floriano L. Mozuela, Eutropio A. Ochavillo, Eusebio M. Ong Sotto, Antonio L. Ordoveza, Pablito G. Picardal, Rosalina A. Posadas, Epitacio C. Punzalan, Florendo C. Quebral, Protacio V. Quiamco, Juan K. Quijano Jr., Vivencio L. Quinon, Aquilino G. Ramos, Benicio A. Revilla, Celestino T. Rivera, Lolita C. Rivera, Cleofas C. Rodriguez, Vicente I. Ruma, Sotero S. Salac, Salvador J. Salandanan, Renato E. Sanchez, Angelito R. Sandoval, Pedro D. Sangatanan, Enrique A. Sindico, Angel V. Sobrepeña, Jesus C. Sta. Iglesia, Democrito K. Talian, Bienvenido P. Tamayo, Rodolfo G. Tamondong, Augusto C. Torralba, Bernardo G. Valdez, Rodrigo B. Valdez, Ramon V. Valmayor, Tomas M. Vejerano, Bonifacio D. Villanueva, Oscar C. Villaseñor.

Graduates with the degree of Bachelor of Science in Home Technology were Conchita N. Buedad, Carmelita U. Casanova, Gloria C.

Elauria, Milagros A. Gonzalvo, Angeles O. de Guzman, Milagros E. Jorda, Lourdes N. Laurel, Justina O. Mabesa, Julieta L. Maligaya, Rosalinda J. Martinez, Socorro F. Olaivar, Alicia A. Paelmo, Delfina M. Torreta, Erlinda A. Tucay.

Dean Leopoldo B. Uichanco emplaned for Europe on April 5 on a five-month travel-survey tour under the sponsorship of the Foreign Operations Administration. He will also represent the National Research Council of the Philippines in two conferences to be held in Milan, Italy: the fifth session of the convention of the National Research Council of Italy, April 12 to 14; and the first meeting of directors of national research centers under UNESCO sponsorship, April 14 to 19.

From Milan Dean Uichanco will proceed to Zurich, Paris, Spain, Amsterdam, and London. In the United States he will stay for short periods in Harvard, Cornell, Florida, Purdue, and California universities. He will also visit plant and animal laboratories specially where tropical species are being used. On the way home, he will inspect the sugar and pineapple plantations in Tokyo and Hawaii.

Philippine underproduction and US overproduction was the subject of Dr. Richard Bradfield's talk at the 20th initiation exercises of the Society for the Advancement of Research held at the College February 24.

Dr. Bradfield, consultant and board member of the Rockefeller Foundation, is on a year's leave from Cornell University where he heads the agronomy department. He is here on a survey study of the Far East in connection with the plan of the Foundation to expand its operation in this area.

Vegetable Production Day, the sixth extension field day project sponsored jointly by the College and the Bureau of Agricultural Extension, was celebrated on March 26. Truck and home gardeners, 4-H and rural improvement club members, homemakers, and the general public attended.

The morning schedule included talks by vegetable experts and lecture demonstrations by agronomy staff members on choice of varieties, irrigation, and crop management.

Afternoon exhibits featured vegetable displays and tools and field machinery for culture, seed preservation, and disease, weed, and insect control. Specific problems were answered at the open forum by panel members from the Bureaus of Agricultural Extension, Plant Industry, and Public Schools, Institute of Nutrition, Araneta Institute of Agriculture, and UP College of Agriculture.

Dr. Gerardo O. Ocfemia flew to the United States on April 24 to accept a fellowship granted him by the John Simon Guggenheim memorial foundation for a period of eight months ending December 15 of this year.

He will work with Dr. Francis O. Holmes of the Rockefeller Institute for Medical Research in New York City on insect transmission of virus diseases. He will concurrently serve as visiting investigator for the institute.

The sugar technology curricula, types A and B, were restored to the instructional program of the College effective April. Type A curriculum is offered to sugar chemistry majors, type B to sugar engineering majors. Both five-year courses lead to the degree of bachelor of science in sugar technology and are based on the completion of the common first year of the agricultural curriculum with an average grade of 2.5 or better. The last semester of the fifth year is devoted to sugar mill practice of one milling season in an approved sugar factory.

Martin S. Celino, '29, associate research professor in plant pathology, topped the technologist examination given by the civil service bureau last year.

A paper on chromatographic analysis of the organic and amino acids of abacá by A. R. Carangal Jr., A. M. Catambay, and E. B. Manzanilla was presented at the 231st meeting of the Los Baños Biological Club on February 25. Types of laying houses for poultry farms were also discussed in a second paper by F. M. Fronda and A. C. Campos.

Dr. Paul Work, who returned to Cornell in May, was replaced by Dr. Thomas Lenoir York, graduate of the North Carolina State College and of Cornell University. Dr. Work will be with the College as assistant professor of vegetable crops and plant breeding.

College faculty members and two students were admitted as active members of the Phi Sigma biological honor society at the 6th initiation rites held at the UP social hall. Inducted were Dr. Valeriano C. Calma, Amado C. Campos, Emerita B. de Guzman, Dr. Miguel Manresa Jr., Dr. Faustino T. Orillo, Juan V. Pancho, Dr. Dioscoro L. Umali, Dr. Getulio B. Viado, Leo C. Rimando, Fernando A. Bernardo, and Rodolfo M. Ela.